PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US2004/036178	01.11.2004	30.10.2003

International Patent Classification (IPC) or both national classification and IPC
C12Q1/68

Applicant
NORTH CAROLINA STATE UNIVERSITY

1. This opinion contains indications relating to the following items:

   - ☒ Box No. I Basis of the opinion
   - ☒ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

   If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

   If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

   For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US2004/036178

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material:

      ☐ in written format

      ☐ in computer readable form

   c. time of filing/furnishing:

      ☐ contained in the international application as filed.

      ☐ filed together with the international application in computer readable form.

      ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☐ The following document has not been furnished:

   ☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-50
	No: Claims	
Inventive step (IS)	Yes: Claims	1-50
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-50
	No: Claims	

2. Citations and explanations

   see separate sheet

Reference is made to the following documents:

D1: Lowe et al. 'Laser-induced temperature jump electrochemistry on gold nanoparticle-coated electrodes' (26.11.2003) JOURNAL OF THE AMERICAN CHEMICAL SOCIETY (125):14258-14259

D2: Park et al. 'ARRAY-BASED ELECTRICAL DETECTION OF DNA WITH NANOPARTICLE PROBES' (22-02-2002) SCIENCE, 295 (22):1503-1505:

D3: US2003/0044805 (06.03.2003) NANOSPHERE, INC.

D4: KAI E. ET AL 'Detection of PCR products of Escherichia coli O157:H7 in human stool samples using surface plasmon resonance (SPR)' (2000) FEMS IMMUNOLOGY AND MEDICAL MICROBIOLOGY, 29:283-288

RE Item II

D1 is published on 23.11.2004. Although D1 does not constitute prior art within the meaning of Rule 64.1(b) PCT, it appears to disclose all the features of independent claim 1. The priority document pertaining to the present application was not available at the time of establishing the present communication. Hence, it is based on the assumption that all the claims enjoy priority rights from the filing date of the priority document. If it later turns out that this is not correct, D1 could become relevant to assess whether the claimed subject matter satisfy the criteria set forth in Art. 33(1) PCT.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Clarity

According to the present wording of the claims, the method of claim 1
Dependent claim 15 refers to the method of claim 1, wherein the detection . However,

in the method of claim 1 no detection probe is used.

Dependent claim 28 relates to a method wherein a plurality of different capture probes are used. Dependent claim 29 relates to the method of claim 28 wherein each attachment point of the array is separately exposed to light. However, since it appears that a separate exposition to light is the only feasible way to perform the method of dependent claim 25, said feature should be included in claim 29 and not formulated in a different dependent claim.

The terms *rastering* and *sacrificial electron donor* used in dependent claims 31 and 32, respectively, are unclear, rendering the scope of protection of said claims unclear.

The feature of dependent claim 45, namely that 'the nanoparticle is attached to the target sequence', is already present in the independent claim to which claim 45 refers back. This redundancy of features renders unclarity to the scope of protection of the claims.

According to page 11, lines 3-5 in the description, in the method of claim 1, the electric changes are the result of the laser-induced temperature jumps involving nanoparticles plasmon excitations. However, according to other parts of the description, i.e. page 15, first full paragraph, "some embodiments (...) are free of the use of a target analyte attached to a conductive support and/or a nanoparticle comprising a photoelectrochemically active moiety...". The above contradiction renders unclear the scope of protection of the claims.

It is clear from the description on page 10, lines 20-24, that the nanoparticles used in the method of claim 1 must have plasmon resonances in the region of the light used. Since independent claim 1 does not contain this feature it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

Should the above-mentioned lack of clarity objections be overcome, the following opinion on novelty and inventive step for the subject-matter of claims 1-50 would apply:

Novelty

D2 and D3 disclose an array-based electrical detection of DNA with nanoparticle probes. In said method DNA target molecules are detected using an array of capture probes located between two minielectrodes. Gold nanoparticle-labelled detection probes are hybridised to the immobilised target DNA, resulting in change in the conductivity between the electrodes (cf. page 1197, Box 1; paragraph [0024], respectively).

D4 discloses a method for detecting PCR products using a surface plamon resonance (SPR) BIACON device and peptide nucleic acid as sensor probes (cf. abstract and fig. 1).

Claims 1-50 are, hence, novel over cited prior art.

Inventive step

Document D2 is considered to represent the most relevant state of the art for the method of independent claim 1. D2 discloses a method to identify DNA target molecules. In said method the target nucleic acids are hybridised to an array of capture probes located between two minielectrodes. Gold nanoparticle-labelled detection probes are hybridised to the immobilised target DNA, resulting in change in the conductivity between the electrodes (cf. abstract).

The subject-matter of independent claim 1 differs in that the nanoparticle is exposed to light having a wavelength absorbed by the nanoparticle.

The technical effect of the method of claim 1 would be the identification of very low concentrations, in the order of 100fM, of DNA molecules in a sample.

The problem to be solved by the subject matter of claim 1 may therefore be regarded as the provision of a more sensitive detection method.

The solution would be to irradiate the hybridisation complex with light and detect the electrical signal.

This solution can be considered as involving an inventive step for the following reasons: cited prior art documents D2 and D3 disclose the use of nanoparticles to detect DNA molecules, based on a change in conductivity due to the nanoparticles localise in the electrode gap and the silver deposition. Cited prior art document D4 discloses a method to detect DNA molecules based on surface plasmon resonance (SPR). In said method, the target nucleic acid is detected due a change in the refractive index at the surface, resulting from the SPR (cf. fig. 1). However, none of the cited prior art documents discloses the possibility of using light-induced temperature electrochemistry, associated with SPR, and nanoparticles to detect the presence of a target nucleic acid. Consequently, the Examining Division is of the opinion that application of light-induced temperature electrochemistry and nanoparticles in DNA detection methods involves the use of inventive skills.

The present application does meet the criteria of Article 33(1) PCT, because the subject-matter of claim 1-50 does involve an inventive step in the sense of Article 33(3) PCT.